REMARKS

Upon entry of this amendment, Claims 2-13, 19-21, 34, 39, and new Claims 41-50 are pending. Applicants have canceled Claims 1, 14-18, 22-33, 35-38, and 40 without prejudice. Applicants reserve the right to prosecute the subject matter of the canceled claims in future continuing applications.

Applicants have also amended Claims 2-11, 13, 19, 34 and 39 to clarify the subject matter claimed. Claims 2-13 and 19-21 had been withdrawn as being directed to a non-elected invention. Due to the amendments, these claims now depend from claims of the elected invention, and therefore also belong to the elected group.

Applicants submit that all claim amendments are supported throughout the specification. No new matter is introduced. For example, Applicants have amended claim 34 to recite a method for producing a Purkinje/bone marrow-derived heterokaryon. This amendment is supported, for example, by Example 4, pages 49-58 of the specification. Applicants have further amended claim 34 to recite a method comprising administering an agent that mobilizes bone marrow cells instead of reciting "administering a bone marrow cell mobilization therapy." Supported can be found, for example, on page 38, line 4.

Claim 39 has been amended to refer to a Purkinje/bone marrow-derived heterkaryon in order to correct antecedent basis.

Claims 2, 4, 6, 8, 13, 19, and 21 have been amended to depend on claim 34. Claims 2 and 3 have been amended to recite a "neuron deficiency" instead of a "neuronal deficiency" to correct antecedent basis. Claims 5, 6, and 7 have been amended to recite "bone marrow cells" instead of "bone marrow-derived cells" to correct antecedent basis. Claims 10, 11, and 19 "have been amended to recite "agent that mobilizes bone marrow cells" instead of "bone marrow derived cells" in order to depend (indirectly) from claim 34.

Applicants have also added new Claims 41-50 that depend, directly or indirectly, from claim 34 and that are therefore drawn to the elected invention. Support for these new claims is found throughout the specification. New claim 41 is supported on page 50 (lines 2-4), page 51 (lines 2-6), and on pages 53-54 of the specification, for example. New claim 42 is supported at line 25 on page 39 to line 29 on page 40, line 30 on page 49 to line 3 on page 50, lines 26-29 on

page 50, lines 1-11 on page 51, and in Figures 7b and 7c, for example. New Claims 43 and 44 are supported on page 51, lines 7-13, for example. New Claims 45 and 46 are supported on page 37, line 22 – page 38, line 3, for example. New Claims 47-50 are supported on page 38, lines 4-8, for example.

Applicants respectfully request reconsideration in view of the following remarks. Issues raised by the Examiner will be addressed below in the order they appear in the Office Action.

Claim rejections under 35 USC § 112, first paragraph

Claims 34, 39, and 40 remain rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the enablement requirement. The Examiner raised several issues concerning enablement, and Applicants hereby address each issue separately below.

(1) Regarding alleged no demonstration of therapeutic efficacy

The Examiner argues that the state of the art was (at the time of the instant priority date) and remains not enabling for a therapeutic use for ameliorating any symptom of a neuronal disorder. In response to Applicants' remarks on Sigurjohnsson *et al.*, the Examiner states "at such a low rate of neuronal differentiation, and uncertainty of neuron phenotype and function *in vivo*, any therapeutic effect of mobilized BMDCs on treating a neuronal deficiency would be remote at the time of instant filing date" (Office Action, page 3).

Applicants respectfully disagree.

Applicants submit that this issue relates to *in vitro* / *in vivo* correlation. Pursuant to MPEP 2164.02:

> "Correlation" as used herein refers to the relationship between *in vitro* or *in vivo* animal model assays and a disclosed or a claimed method of use. An *in vitro* or *in vivo* animal model example in the specification, in effect, constitutes a "working example" if that example "correlates" with a disclosed or claimed method invention. ... if the art is such that a particular model is recognized as correlating to a specific condition, then it should be accepted as correlating unless the examiner has evidence that the model does not correlate. Even with such evidence, the examiner must weigh the evidence for and against correlation and decide whether one skilled in the art would accept the model as reasonably correlating to the condition. *In re Brana*, 51 F.3d 1560, 1566, 34 USPQ2d 1436, 1441 (Fed. Cir. 1995) (reversing the PTO decision based on finding that *in vitro* data did not support *in vivo* applications).

...

A rigorous or an invariable exact correlation is not required, as stated in *Cross v. Iizuka*, 753 F.2d 1040, 1050, 224 USPQ 739, 747 (Fed. Cir. 1985):

[B]ased upon the relevant evidence as a whole, there is a reasonable correlation between the disclosed *in vitro* utility and an *in vivo* activity, and therefore a rigorous correlation is not necessary where the disclosure of pharmacological activity is reasonable based upon the probative evidence. (Citations omitted.)

Therefore, controlling case law does not require Applicants to demonstrate actual efficacy (such as ameliorating at least one disease symptom) in human patients in order to enable the claimed method. All that is a required is a reasonable correlation between the animal model and the claimed disease condition. In contrast, the Examiner appears to require Applicants to demonstrate actual efficacy in human patients in order to satisfy the enablement requirement, such a strict requirement is inconsistent with the relevant case law requiring merely "reasonable" correlation of the *in vitro* or *in vivo* animal working examples with the claimed method.

Nevertheless, solely to expedite prosecution, Applicants have amended the claims to recite a method for "producing a Purkinje/bone marrow-derived heterokaryon, comprising administering an agent that mobilizes bone marrow cells." Applicants submit that the amended claims no longer recite "treating a neuronal deficiency" or " ameliorating at least one symptom of the neuronal deficiency," and there is no need to demonstrate any therapeutic efficacy or any actual ameliorated symptoms. Therefore, at least the amended claims are fully enabled.

(2) Regarding specific agents and conditions for the use of agents to mobilize bone marrow cells

The Examiner further argues that "the specification fails to describe any specific agent or condition under which and (sic) using such agent the bone marrow cell mobilization therapy can be carried out, and it fails to teach what kind of effect in the brain tissue one is to expect upon the stem cell mobilization, and hence there is a failure to meet the enablement requirement." (p. 5 of the Office Action). Applicants respectfully traverse.

Contrary to the Examiner's assertion, the instant specification describes many specific agents that can be used to mobilize bone marrow cells.

For example, on page 37, lines 24-26, the specification teaches that "[t]he use of **granulocyte colony stimulating factor (G-CSF)** for bone marrow cell mobilization is well

known (see, e.g., Chao *et al.*, 1993, Blood 81(8):2031-2035)." The specification also teaches the use of **GM-CSF, Flt-3 ligand, MIP.alpha, anti-VLA-4 antibodies, and anti-VCAM-1 antibodies** (page 37, line 27 – p. 38, line 8) for bone marrow cell mobilization, and the corresponding references enable the use of such bone marrow cell mobilization agents. Some of these known bone marrow cell mobilization agents are described and claimed in issued U.S. patents, which are presumed to be valid and enabled. Applicants wish to remind the Examiner that clear and convincing evidence is required to prove invalidity of issued claims.

Regarding specific **conditions** under which bone marrow cells can be mobilized, the instant specification provides detailed description on, for example, page 37 line 28 – page 38 line 8. The specification further describes the expected **effects** that bone marrow cell mobilization therapy would have on the brain. The formation of heterokaryon Purkinje neurons is expected, based on the evidence in Example 4, for instance.

In addition to the explicit teachings of the instant specification, Applicants further submit that it was well-known in the art that bone marrow cell mobilization therapy (or administration of an agent that mobilizes bone marrow cells) results in the movement of bone marrow cells into the circulation. Therefore, administering an agent that mobilizes bone marrow cells can serve as a substitute for injection of bone marrow cells. Since Applicants have already demonstrated that injected bone marrow cells can navigate the blood circulation and find their way to the CNS (and eventually form Purkinje/bone marrow-derived heterokaryon), there is no scientific reason to doubt that mobilized bone marrow cells cannot do the same.

For example, the specification provides G-CSF treatment as an exemplary bone marrow cell mobilization therapy. Applicants further cited, in the previous Office Action response, a few additional publications to support Applicants' position.

Specifically, Zohlnhofer *et al.* (JAMA. 2006 Mar 1;295(9):1003-10, Exhibit 1 in the prior Response) demonstrates that G-CSF is an effective mobilization treatment in humans. This post-filing evidence verifies the teaching of the instant specification regarding using G-CSF to mobilize bone marrow cells in human.

Applicants also noted that in mice, G-CSF increased peripheral blood pluripotent hematopoietic stem cells by 250-fold (abstract, Bodine *et al.* 1994 Blood (84): 1482-1491, Exhibit 2 in the prior Response). This study shows that, similar to directly injecting bone

marrow cells into a recipient, bone marrow cell mobilization agents such as G-CSF can be used to dramatically increase the amount of HSC in peripheral blood, thereby achieving the same result of direct injection.

Applicants further cited Orlic *et al.* as additional evidence that bone mobilization treatment can be a substitute for injection of or treatment with bone marrow cells. Orlic *et al.* demonstrated that bone mobilization treatment achieved similar clinical effects as injected bone marrow cells in promoting myocardial repair (2001 PNAS (98): 10344-10349, Exhibit 3 in the prior Response).

The Examiner states that two of the afore-mentioned references were published after the instant application's priority date. However, Applicants submit that the post-filing evidence confirms that the instant specification is enabled as of the filing date of the instant application, because they describe the inherent property of G-CSF when used as a bone marrow mobilization agent. One of skill in the art does not need to rely on the information in these references to practice the claimed invention.

Applicants further submit that bone marrow cell mobilization therapies were widely practiced in the art at the time of the instant priority date, and it was widely known that such therapies result in the movement of bone marrow cells from the bone marrow into the circulation. For example, as early as 1988, Dürhsen *et al.* demonstrated that recombinant G-CSF treatment in humans resulted in a "pronounced increase in the number of circulating progenitor cells and, in most patients, a slight reduction in the frequency of bone marrow progenitor cells" (page 2079, second column of Dürhsen *et al.* 1988, Blood (72): 2074-2081, emphasis added, enclosed as Exhibit 1). Applicants also provide Lane *et al.* (1995 Blood (85): 275-282, enclosed as Exhibit 2) and Lemoli *et al.* (1997 Blood (89): 1189-1196, enclosed as Exhibit 3) as evidence that G-CSF and GM-CSF are successful bone marrow mobilization therapies that result in the circulation of bone marrow-derived cells. Indeed, Lemoli *et al.* compare certain characteristics of G-CSF-mobilized peripheral blood stem cells with bone marrow cells and conclude "In summary, our results indicate that CD34+ cells mobilized into PB [peripheral blood] of normal donors by glycosylated G-CSF (lenograstim) can be considered equivalent to their BM counterparts, on a cell per cell basis, in their content of both committed and very primitive hematopoietic progenitors capable of repopulating the hematopoietic tissue" (page 1195, last paragraph). Moreover, other treatments in addition to G-CSF and GM-CSF for bone marrow

mobilization were known at the time of the instant priority date (e.g., Sudo *et al.* 1997 Blood (89) 3186-3191, enclosed as Exhibit 4, and Laterveer *et al.* 1996 Blood (87):781-788, enclosed as Exhibit 5).

Accordingly, bone marrow cell mobilization therapy can be a substitute for injection or infusion with bone marrow-derived cells, as both mobilization treatment and injection result in the circulation of bone marrow-derived cells. Since the specification teaches using bone marrow cell mobilization therapy (e.g., page 37 line 22-page 38, line 12) and demonstrates that bone marrow cells in circulation can form Purkinje/bone marrow-derived heterokaryons, the claimed invention is fully enabled. Applicants respectfully request reconsideration and withdrawal of the rejection.

(3) Regarding the alleged silence on the effect of the mobilized bone marrow cells on neuronal system

The Examiner argues that the publications referred to in the prior Response do not address the effect of mobilized bone marrow cells on the neuronal system (Office Action, page 5). However, as stated in the prior Response, these publications serve to provide support for the effectiveness of agents that mobilizes bone marrow cells, rather than for any effect of bone marrow cells on the neuronal system. As argued above, Applicants have already demonstrated in the instant application that injected bone marrow cells can navigate the blood circulation and find their way to the CNS (and eventually form Purkinje/bone marrow-derived heterokaryon). Since the bone marrow mobilization agents, such as those used in the cited publications, can effectively mobilize bone marrow cells, these agents function as substitutes for direct injection of bone marrow cells into the circulation. Thus, there is no scientific reason to doubt that the mobilized bone marrow cells cannot be as effective in the neuronal system as the injected bone marrow cells.

Indeed, the novel outcome of bone mobilization therapy in the neuronal system, as taught by the instant specification, is the subject of the presently claimed invention.

(4) Regarding the alleged unpredictable effect of administering bone marrow cell mobilization agents (as opposed to direct injection)

The Examiner also alleges that the specification fails to teach whether a bone marrow cell mobilization therapy would boost relocation of bone marrow cells to the brain and cause their differentiation into neurons in a sufficient quantity and quality.

In addition, the Examiner states, on page 6 of the Office Action, that "the observed neuronal differentiation occurred in the circumstance where the recipient received lethal irradiation and BM cell transplantation, where the instant claims are drawn to administering cytokines G-CSF/GM-CSF without any cell transplantation."

Therefore, the Examiner concludes that it is unpredictable whether administering a factor such as G-CSF would cause neuronal differentiation.

Applicants respectfully disagree.

Similarities between BM cell transplantation and administering an agent that mobilizes bone marrow cells have been discussed above. The Examiner has not provided a sound reason to explain why mobilized bone marrow cells would behave differently from the injected bone marrow cells. Since Applicants have already demonstrated the production of Purkinje/bone marrow-derived heterokaryons *in vivo* following administration of bone marrow cells (see, e.g., Example 4), there is no reason to doubt that mobilized bone marrow cells could not do the same.

Applicants hereby provide additional evidence, in the form of a Rule 132 Declaration by inventor Helen Blau, to further support the enablement of the claimed invention.

Specifically, Figure 1 the Declaration shows that the progeny of a single hematopoietic stem cell (HSC) can form heterokaryons with Purkinje neurons. In that experiment, a single HSC cell was isolated from the bone marrow (BM) of an EGFP transgenic mouse (thus the HSC cell and all its progeny are labeled by green fluorescent). The isolated single HSC cell was then transplanted with 10^6 helper cells into a lethally irradiated wild-type mouse. Figure 1b shows a high power laser-scanning confocal image from a cerebellar sagittal section of the mouse transplanted with the single HSC. Immunohistochemistry with specific antibodies demonstrates the presence of GFP in a single cerebellar heterokaryon (green) co-expressing Calbindin (red) in Purkinje neurons. This experiment demonstrates the ability of a single injected HSC to produce a fusion with Purkinje neurons to form cerebellar heterokaryons.

If a single injected HSC and its progeny can find their way to the CNS and fuse with the Perkinji cells there, it is hard to imagine how a bone marrow cell mobilization agent (such as G-

CSF), which reportedly can lead to a 250-fold increase of peripheral blood pluripotent hematopoietic stem cells (see Bodine, *supra*), would fail to boost relocation of bone marrow cells to the brain and cause their differentiation into neurons, as the Examiner has argued.

Regarding the potential artifacts associated with lethal irradiation in BM transplantation, Applicants maintain that the specification teaches that heterokaryon Purkinje neurons can be formed without lethal irradiation. On page 33, line 24 through page 34 line 6, the specification state that a variety of ablative regiments may be used. Ablative therapy may be irradiation, or may be another form of treatment.

Applicants hereby provide direct evidence, in the form of the accompanying Rule 132 Declaration by inventor Helen Blau, to further support this position.

Specifically, Figure 2 of the Declaration demonstrates that fusion and formation of cerebellar heterokaryons is independent of variables associated with bone marrow transplantation, such as lethal irradiation. In the well-established parabiosis model, two female mice (one wild type recipient, and one transgenic mouse that ubiquitously expresses EGFP, the donor) are surgically joined from the olecranon to the knee, such that GFP+ blood from the donor mouse was introduced into the wild-type recipient mouse without exposure to irradiation (Fig. 2a). Parabiotic animals develop a common anastomosed circulatory system within 3-10 days, and reach blood chimeric equilibrium within 7 to 21 days.

Thirty-three such parabionts were created between age matched wild type and GFP+ transgenic mice, and the pairs were analyzed at various time-points thereafter ranging from 12-54 weeks post surgery. Although few (n=0-2) cerebellar heterokaryons were detected in parabionts after 12 weeks as parabionts (data not shown), a significant number (n=12-43) were detected after 20-26 weeks as parabionts. (Fig. 2b). All heterokaryons were binucleated and expressed the red Purkinje neuron-marker Calbindin.

These data demonstrate that formation of cerebellar heterokaryons occurs in the absence of variables associated with the BMT procedure, such as lethal irradiation. In other words, HSCs not introduced by cell transplantation can also fuse with Purkinje neurons in the absence of lethal irradiation. The methods that worked in combination with lethal irradiation also work without lethal irradiation.

Thus, the specifications teach that administering a factor that mobilizes bone cells causes formation of heterokaryon Purkinje neurons, with or without irradiation, and with or without direct cell transplantation.

Claim rejections under 35 U.S.C. § 112, second paragraph

Claims 34, 39, and 40 remain rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

The Examiner argues that the recitation of a "bone marrow cell mobilization therapy" does not clearly set forth a step for the therapeutic regimen. The Examiner states that "method claims need not recite all operating details but should at least recite positive, active steps so that the claims will set out and circumscribe a particular area with a reasonable degree of precision and particularity and make clear what subject matter that claims encompass as well as make clear the subject matter from which others would be precluded" (Office Action, pages 7-8).

Applicants respectfully traverse. Applicants maintain that a skilled artisan would know, based on the skill in the relevant art, what steps constitute "administering a bone marrow cell mobilization therapy." Thus the metes and bounds of the claims as originally filed are clear.

Nevertheless, solely to expedite prosecution, and not in acquiescence to the Examiner's remarks, Applicants have amended the claims to recite a method comprising administering an agent that mobilizes bone marrow cells. Thus, even according to the Examiner's own standard, the amended claims recite a "positive, active" step of administering an agent. Accordingly, this rejection is overcome. Applicants respectfully request reconsideration and withdrawal of the rejection.

CONCLUSION

In view of the foregoing, Applicants believe the pending application is in condition for allowance.

Applicants believe no fee other than those authorized in the amendment transmittal (filed concurrently herewith) are due with this response. However, if any other fee is due, please charge our Deposit Account No. **18-1945**, from which the undersigned is authorized to draw under Order No. **SUPP-P01-011**.

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Respectfully submitted,

By_____

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